Design and Development of Novel Formulations for Prophylaxis and Treatment of Cold Injury at High Altitude

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Introduction

Traditionally many active pharmaceutical ingredients have been established to combat problems associated with extreme environments, particularly high altitude sickness and other problems. However, despite several advancements, few formulations have conclusively been proved in modern medicine to aid in physiological adaptation. Traditional medicine has been used for last several years by natives to get acclimatized in extreme environments, including high altitude. One of the best therapies can be established, if the concern of drug delivery associates with specific area of the disease targeting. Many of the API of synthetic origin such as Acetazolamide (Diuretic) and Dexamethasone (corticosteroid) shows therapeutic efficacy in the higher altitude problems but exposure to these molecules also produces some side effects. Based on the leads obtained in DIPAS with medicinal mushrooms viz., *Ganoderma lucidum* and *Cordyceps sinensis sps.* it is conceptualized to design pharmaceutical formulations for the lead compounds from natural sources for Physiological Adaptation to Extreme Environments.

With aid of all research plan, we suggest to develop some of the pharmaceutical dosage form, which may be either semisolid preparation, colloidal system or nanoparticles drug delivery system with the already established lead compounds as DIP-G-Fit/DIP-C-Fit.

Rationale of studies

Allopathic medicine already had a large scale therapeutic background but instead of these medicine a complete gain of therapeutic efficacy is a questionable subject due to their severe inverse effect on health upon prolonged treatment.

While treatment with natural substances is generally regarded as safe (GRAS) and accepted by the both patient and therapist in both aspects of prophylaxis as well as therapy.

In recent advancement of pharmaceutical preparations, natural products are gaining more attention to treat disease due to their less toxicity, high potential in therapeutic system. Working with a natural extract to formulate a pharmaceuticals agent is quite challenging aspect in drug

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development, however, there is evidence of improved efficacy of natural products and this gives hope to our study

What are the possible complications arises in higher altitude?

In case of higher altitude, most serious problems include pulmonary edema, cerebral edema and thrombo-embolism. Pulmonary edema is manifested as severe breathlessness cough and frothy expectoration. If someone has staggering gait like drunk, headache, vomiting and sleepiness it could be due to cerebral edema. Thromboembolism can lead to acute pain in abdomen due to splenic infarction or intestinal ischemia. Sudden visual loss in one eye, usually transient, have been frequently encountered. Rarely lung infarction and venous sinus thrombosis in brain occur as severe consequences of thromboembolism. Some cases of myocardial infarction may also be the result of high altitude. Rise of blood pressure by 20-30 mm of Hg diastolic blood pressure is a common observation.

In higher altitude where almost 150 post of Indian army with the command of 10,000 soldiers has been posted to protect our nation. These soldiers are trained with all modern training and technique but instead of this many of the surveys reveals fighting with those environment are not easy as we assume about them. Many of the common problems associated with health risk have been observed in those soldiers who are posted at very higher altitude eg. Leh and Siachen. One of the common problem they face in higher altitude is Cold injuries (like frostbites), it has been reported that if a soldier comes in the touch with their refile with a necked hand, the intact skin get frozen down within 15 sec. and mostly those condition are unrecoverable in nature. Frostbite is a localized freezing injury known to plague travelers, mountaineers, soldiers, scientists, and the general population who are exposed to cold. Severity of this condition only limit to the treatment with general medicine as aspirin and ibuprofen treatment etc. but to protect our soldiers who protect our nation we need to find out the solutions with total care of these conditions.

With the aim of this objective we are suggesting an approach for a topical formulation which comes with the ingredient of herbal/natural phyto-constituents with the aid of a rejuvenate therapy for dermatological problem occurs in higher altitude.

Objective of the proposed studies

1. *Design and development of novel formulations for the prophylaxis and treatment of cold injury using the Quality-by-Design (QbD) approach.*
2. *To evaluate the safety and efficacy of the developed formulations on the Sprague Dawley rat model (in vivo).*
3. *To investigate the mechanistic aspect of the developed formulation on the cold injury rat model*

Methodologies/Strategies to be adopted

1. **Characterization of the phytoconstituents:** FTIR, NMR, UV, DSC, HPTLC, HPLC etc.
2. **Development of the study protocols as per the requirement of the higher altitude maladies:** Antioxidant activity of the obtained compound As DPPH activity, ABST, FRAP, TPC and TFC
3. **Pre-formulation study of the model drug and analytical method development by means of using HPLC or UV- Visible spectroscopy.** The following pre-formulation study will be carried out during the study as- determination of the melting point, solubility, pH, partition coefficient, and development of the standard calibration curve, characterization of the pure drug by DSC, ATR-FTIR.
4. **Physico-chemical characterization of topical formulation:** Morphological and physicochemical characterization of the developed topical formulation.
5. **Drug-drug and drug-excipient compatibility studies:** By means of Fourier Transform Infrared Spectroscopy (FTIR) & Differential scanning calorimeter (DSC).
6. **Determination of drug entrapment efficiency, drug loading and percentage yield:** The entrapment efficiency will be determined by the separation of drug loaded

pharmaceutical dosage form, from the aqueous medium containing non-associated drug by ultracentrifugation.

7. ***In-vitro* drug release studies**: The *in-vitro* drug release studies will be carried out by using Franz's diffusion cell.

8. **Cell viability study:** The MTT assay will be carried out for the pharmaceutical dosage form.

9. **Method validation, evaluation and testing of phytoconstituents: *in silico*, *in vitro* and *in vivo* models.**

10. **Stability studies:** The stability studies will be carried out according to ICH guidelines.

11. **Statistical analysis:** Optimization of the data will be done by using appropriate software's.

12. **Statistical validation:** The data will be presented as mean ± S.D. the data on *in-vitro* drug release from the prepared pharmaceutical dosage forms will be compared using similarity factor (f2), ANOVA, Student't' test, etc.

Expected outcomes

With the approaches of the proposed study and on the basis of some earlier findings which is reported in many articles, a novel development of the herbal agents either in topical drug delivery system or oral delivery system with the potency of combating life threatening diseases of the higher maladies can be formulated. So that we can replace the synthetic available product with the natural origin compound and can decrease other health risk issue which arises due to the long term treatment with the synthetic medicine.

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